

Amendment to the Claims

1. (Currently amended) A non-naturally occurring single chain protein comprising: i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule, said binding domain polypeptide comprising a heavy chain variable region, ~~said heavy chain variable region comprising~~ containing an amino acid substitution ~~or deletion~~ corresponding to amino acid position 11 of a heavy chain variable region; ii) a second polypeptide comprising ~~an altered wild type IgG1 immunoglobulin hinge region-a connecting region~~ attached to said first polypeptide, wherein the altered hinge region has only the third cysteine of a wild type IgG1 hinge region or has only the first and third cysteines of a wild type IgG1 hinge region; and iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity.

2. (Previously presented) The protein of claim 1 wherein said binding domain polypeptide is a single chain Fv.

3. (Currently amended) The protein of claim 1 wherein the amino acid substitution ~~or deletion~~ in said heavy chain variable region is effective to increase expression or stability of said protein relative to a protein without said ~~deletion or~~ substitution.

4. (Previously presented) The protein of claim 1 wherein said binding domain polypeptide comprises an immunoglobulin light chain variable region polypeptide and an immunoglobulin heavy chain variable region polypeptide.

5. (Previously presented) The protein of claim 4 further comprising a second binding domain polypeptide capable of binding a second target molecule, said second binding domain polypeptide comprising an immunoglobulin light chain variable region polypeptide and an immunoglobulin heavy chain variable region polypeptide.

6. (Previously presented) The protein of claim 5 wherein the first target molecule and the second target molecule are different.

7. (Previously presented) The protein of claim 5 wherein the first target molecule and the second target molecule are the same.

8. (Currently amended) The protein of claim 1 further comprising ~~one or more~~ additional amino acid substitution ~~corresponding to~~ at amino acid ~~positions~~ position 9, 10, 12, 108, 110, or 112 in said heavy chain variable region.

9. (Canceled)

10. (Currently amended) The protein of ~~claim 9~~ claim 1 wherein the amino acid substituted for the amino acid at position 11 of the single chain Fv heavy chain variable region is selected from the group consisting of serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, and histidine.

11. (Canceled)

12. (Currently amended) The protein of ~~claim 9~~ claim 1 wherein where leucine is replaced by serine at position 11.

13. (Canceled)

14. (Previously presented) The protein of claim 12 having an increased recombinant expression or stability relative to said protein not having an amino acid substitution at position 11.

15. (Previously presented) The protein of claim 14 wherein the expression of said protein having an amino acid substitution at position 11 is 10-100 fold greater than said protein without a substitution at position 11.

16. (Previously presented) The protein of claim 14 wherein said expression is in mammalian cells.

17. (Canceled)

18. (Currently amended) The protein of claim 1 wherein said binding domain polypeptide is a single chain Fv and said binding domain polypeptide comprises a light chain variable region, wherein said light chain variable region has ~~an~~ one additional amino acid

~~deletion or substitution at one or more of amino acid positions~~ position 12, 80, 81, 83, 105, 106, ~~and~~ or 107.

19. (Currently amended) The protein of claim 18 wherein the one additional amino acid substitution is at position 106 ~~has been substituted or deleted~~.

20. (Previously presented) The protein of claim 2 wherein said binding domain polypeptide binds to a tumor antigen.

21. (Previously presented) The protein of claim 2 wherein said binding domain polypeptide binds to an antigen on an immune effector cell.

22. (Previously presented) The protein of claim 2 wherein said binding domain polypeptide binds to a cancer cell antigen.

23. (Previously presented) The protein of claim 22 wherein said cancer cell antigen is a surface antigen.

24. (Previously presented) The protein of claim 22 wherein said cancer cell antigen is an intracellular antigen.

25. (Previously presented) The protein of claim 1 wherein said binding domain polypeptide binds to a B cell antigen.

26. (Previously presented) The protein of claim 25 wherein said B cell antigen is selected from the group consisting of CD19, CD20, CD22, CD37, CD40, CD80, and CD86.

27. (Previously presented) The protein of claim 2 wherein said single chain Fv binds to a B cell antigen.

28. (Previously presented) The protein of claim 27 wherein said B cell antigen is selected from the group consisting of CD19, CD20, CD22, CD37, CD40, CD80, and CD86.

29. (Previously presented) The protein of claim 28 wherein said single chain Fv is selected from the group consisting of HD37 single chain Fv, 2H7 single chain Fv, G28-1 single chain Fv, and 4.4.220 single chain Fv.

30. (Previously presented) The protein of claim 2 wherein said single chain Fv is selected from the group consisting of HD37 single chain Fv, 2H7 single chain Fv, G28-1 single chain Fv, FC.sub.2-2, UCHL-1, 5B9, L6, 10A8, 2e12, 40.2.36, G19-4, 1D8, and 4.4.220 single chain Fv.

31. (Previously presented) The protein of claim 1 wherein said binding domain polypeptide is an scFv that binds to a B cell differentiation antigen.

32. (Previously presented) The protein of claim 31 wherein said B cell antigen is selected from the group consisting of CD19, CD20, CD22, CD37, and CD40.

33. (Previously presented) The protein of claim 1 wherein said binding domain polypeptide binds to a target selected from the group consisting of CD2, CD3, CD4, CD5, CD6, CD8, CD10, CD11b, CD14, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD28, CD30, CD37, CD40, CD43, CD50 (ICAM3), CD54 (ICAM1), CD56, CD69, CD80, CD86, CD134 (OX40), CD137 (41BB), CD152 (CTLA-4), CD153 (CD30 ligand), CD154 (CD40 ligand), ICOS, L6, B7-H1, and HLA class II.

34. (Previously presented) The protein of claim 1 wherein said protein is capable of forming a complex comprising two or more of said proteins.

35. (Previously presented) The protein of claim 34 wherein said complex is a dimer.

36. (Previously presented) The protein of claim 1 wherein said protein is a monomer.

37. (Previously presented) The protein of claim 1 coupled to a drug, toxin, immunomodulator, polypeptide effector, isotope, label, or effector moiety.

38. (Previously presented) The protein of claim 1 wherein said immunological activity is selected from the group consisting of antibody dependent cell-mediated cytotoxicity, complement fixation, induction of apoptosis, induction of one or more biologically active signals, induction of one or more immune effector cells, activation of cellular differentiation, cellular activation, release of one or more biologically active molecules, and neutralization of an infectious agent or toxin.

39. (Previously presented) The protein of claim 38 which is capable of induction of biologically active signals by activation or inhibition of one or more molecules selected from the group consisting of protein kinases, protein phosphatases, G-proteins, cyclic nucleotides or other second messengers, ion channels, and secretory pathway components.

40. (Previously presented) The protein of claim 38 which is capable of induction of one or more immune effector cells selected from the group consisting of NK cells, monocytes, macrophages, B cells, T cells, mast cells, neutrophils, eosinophils, and basophils.

41. (Previously presented) The protein of claim 40 wherein said induction of one or more immune effector cells leads to antibody dependent cell-mediated cytotoxicity or the release of one or more biologically active molecules.

42. (Previously presented) The protein of claim 38 which is capable of cellular activation, wherein said activation leads to changes in cellular transcriptional activity.

43. (Previously presented) The protein of claim 42 wherein said cellular transcriptional activity is increased.

44. (Previously presented) The protein of claim 42 wherein said cellular transcriptional activity is decreased.

45. (Previously presented) The protein of claim 38 wherein said one or more biologically active molecules is a protease.

46. (Previously presented) The protein of claim 38 wherein said one or more biologically active molecules is a cytokine.

47. (Previously presented) The protein of claim 46 wherein said cytokine is selected from the group consisting of monokines, lymphokines, chemokines, growth factors, colony stimulating factors, interferons, and interleukins.

48. (Previously presented) The protein of claim 38 which is capable of neutralization of an infectious agent, wherein said infectious agent is a bacterium, a virus, a parasite, or a fungus.

49. (Previously presented) The protein of claim 38 which is capable of neutralization of a toxin, wherein said toxin is selected from the group consisting of endotoxins and exotoxins.

50. (Previously presented) The protein of claim 38 which is capable of neutralization of a toxin, wherein said toxin is an exotoxin selected from the group consisting of anthrax toxin, cholera toxin, diphtheria toxin, pertussis toxin, E. coli heat-labile toxin LT, E. coli heat stable toxin ST, shiga toxin, Pseudomonas Exotoxin A, botulinum toxin, tetanus toxin, Bordetella pertussis AC toxin, and Bacillus anthracis EF.

51. (Previously presented) The protein of claim 38 which is capable of neutralization of a toxin, wherein said toxin is an endotoxin selected from the group consisting of saxitoxins, tetrodotoxin, mushroom toxins, aflatoxins, pyrrolizidine alkaloids, phytohemagglutinins, and grayanotoxins.

52. (Previously presented) The protein of claim 1 wherein said protein is capable of binding to an intracellular target to effect a cellular function.

53. (Previously presented) The protein of claim 1 wherein said binding domain polypeptide comprises a light chain variable region attached to said heavy chain variable region by a binding domain linker, wherein said binding domain linker comprises one or more peptide having a sequence Gly-Gly-Gly-Gly-Ser (SEQ ID NO: 516).

54. (Previously presented) The protein of claim 53 comprising three Gly-Gly-Gly-Gly-Ser (SEQ ID NO: 516) peptides.

55. (Previously presented) The protein of claim 1 wherein said binding domain polypeptide comprises wild type or engineered immunoglobulin variable region obtained from species selected from the group consisting of human, murine, rat, pig, and monkey.

56. (Previously presented) The protein of claim 1 wherein said binding domain polypeptide comprises a humanized immunoglobulin variable region.

57. (Previously presented) The protein of claim 2 wherein said N-terminally truncated immunoglobulin heavy chain constant region polypeptide comprises an IgG CH2

constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide.

58. (Previously presented) The protein of claim 2 wherein said N-terminally truncated immunoglobulin heavy chain constant region polypeptide consist essentially of an IgG CH2 constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide.

59. - 60. (Canceled)

61. (Previously presented) The protein of claim 1 wherein said binding domain polypeptide is a single chain Fv that comprises at least a portion of a human constant region.

62. (Previously presented) The protein of claim 2 wherein said binding domain polypeptide is a single chain Fv that comprises at least a portion of a human constant region.

63. – 70. (Canceled)

71. (Currently amended) The protein of claim 1 wherein said ~~connecting region~~ hinge region comprises ~~a mutated wild-type immunoglobulin hinge region polypeptide comprising~~ no more than one cysteine residue.

72. (Currently amended) The protein of claim 1 wherein said ~~connecting region~~ hinge region ~~is altered so that said protein~~ has a reduced ability to dimerize.

73. - 74. (Canceled)

75. (Currently amended) The protein of claim 1 ~~[[74]]~~ wherein said wild-type hinge region polypeptide is from human IgG1.

76. (Canceled)

77. (Currently amended) A non-naturally occurring single chain Fv protein comprising: i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule, said binding domain polypeptide comprising a heavy chain variable region wherein ~~leucine is replaced by~~ a serine is at position 11 in the first framework region of the heavy chain variable region; ii) a second polypeptide comprising an altered wild type IgG1

immunoglobulin hinge region ~~a connecting region~~ attached to said first polypeptide, wherein the wild type IgG1 hinge region comprises first, second, and third cysteine residues, and a proline, wherein the first cysteine residue is N-terminal to the second cysteine, the second cysteine is N-terminal to the third cysteine, and the third cysteine is N-terminal to the proline residue and wherein the altered hinge region has only the third cysteine or has only the first and third cysteines of a wild type IgG1 hinge region; and iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity.

78. (Currently amended) The protein of claim 77 further comprising a substitution ~~or deletion~~ of the amino acid at position 10 in the first framework region of the heavy chain variable region.

79. (Currently amended) The protein of claim 77 wherein the ~~amino acid~~ substitution serine at position 11 is effective to increase expression or stability of said single chain Fv protein relative to a single chain Fv protein without said ~~deletion or~~ substitution.

80. – 82. (Canceled)

83. (Currently amended) The protein of ~~claim 81~~ claim 77 where said single chain protein comprises a 2H7 single chain Fv binding domain ~~from a 2H7 hybridoma~~, wherein said second cysteine residue is ~~replaced by~~ substituted with serine and said proline residue is ~~replaced by~~ substituted with serine in the ~~connecting~~ hinge region, and wherein said heavy chain constant region comprises CH2 and CH3 domains from IgG₁.

84. (Canceled)

85. (Currently amended) The protein of ~~claim 81~~ claim 77 wherein said single chain protein comprises a 2H7 single chain Fv binding domain, wherein said ~~first, second, and third cysteine residue~~ residues in the ~~connecting~~ hinge region are ~~replaced by~~ is substituted with serine and said proline residue in the ~~connecting~~ hinge region is ~~replaced by~~ substituted with serine, wherein said heavy chain constant region comprises CH2 and CH3 domains from IgG₁ and wherein ~~lysine is replaced by serine at~~ a serine is substituted into position 331 or 322 in said CH2 region.

86. (Currently amended) The protein of ~~claim 81~~ claim 77 wherein said single chain protein comprises a 2H7 single chain Fv binding domain from a ~~2H7 hybridoma~~, wherein in the ~~connecting hinge~~ region said first and second ~~and third~~ cysteine residues are ~~replaced by~~ substituted with serine and said proline residue is ~~replaced by~~ substituted with serine, wherein said heavy chain constant region comprises CH2 and CH3 domains from IgG, and wherein ~~lysine is replaced by serine at~~ a serine is substituted into position 331 or 322 in said CH2 region.

87.-88. (Canceled)

89. (Currently amended) The protein of ~~claim 81~~ claim 77 wherein said single chain protein comprises a 2H7 single chain Fv binding domain, wherein in the ~~connecting hinge~~ region said ~~first, second, and third~~ cysteine residues are ~~replaced by~~ residue is substituted with serine and said proline residue is ~~replaced by~~ substituted with serine, wherein said heavy chain constant region comprises CH2 and CH3 domains from IgG, and wherein threonine is ~~replaced by~~ substituted with asparagine at position 256 in said CH2 region.

90. (Currently amended) The protein of ~~claim 81~~ claim 77 wherein said single chain protein comprises a 2H7 single chain Fv binding domain, wherein in the ~~connecting hinge~~ region said ~~first, second, and third~~ cysteine residues are ~~replaced by~~ residue is substituted with serine and said proline residue is ~~replaced by~~ substituted with serine, wherein said heavy chain constant region comprises 2H7 single chain Fv CH2 and CH3 domains from IgG, and wherein in the CH2 domain arginine is ~~replaced by~~ substituted with glutamine at position 255, threonine is ~~replaced by~~ substituted with asparagine at position 256, proline is ~~replaced by~~ substituted with alanine at position 257, and glutamic acid is ~~replaced by~~ substituted with lysine at position 258.

91. (Currently amended) The protein of ~~claim 81~~ claim 77 wherein said single chain protein comprises a 2H7 single chain Fv binding domain from a ~~2H7 hybridoma~~, wherein in the ~~connecting hinge~~ region said ~~first, second, and third~~ cysteine residues are ~~replaced by~~ residue is substituted with serine and said proline residue is ~~replaced by~~ substituted with serine, wherein said heavy chain constant region comprises CH2 and CH3 domains from IgG₁, and wherein lysine is ~~replaced by~~ substituted with glutamine at position 290 in said CH2 region.

92. (Currently amended) The protein of ~~claim 81~~ claim 77 wherein said single chain protein comprises a 2H7 single chain Fv binding domain from a ~~2H7~~ hybridoma, wherein in the ~~connecting hinge~~ region said ~~first, second, and third~~ cysteine residues are ~~replaced by~~ residue is substituted with serine and said proline residue is ~~replaced by~~ substituted with serine, wherein said heavy chain constant region comprises CH2 and CH3 domains from IgG, and wherein alanine is ~~replaced by~~ substituted with proline at position 339 in said CH2 region.

93. (Canceled)

94. (Currently amended) The protein of ~~claim 81~~ claim 77 wherein said single chain protein comprises a G28-1 single chain Fv binding domain from a ~~G28-1~~ hybridoma, wherein in the ~~connecting hinge~~ region said first and second ~~and third~~ cysteine residues are ~~replaced by~~ substituted with serine and said proline residue is ~~replaced by~~ substituted with serine, and wherein said heavy chain constant region comprises CH2 and CH3 domains from IgG₁.

95. (Previously presented) The protein of ~~claim 81~~ claim 77 wherein said single chain protein comprises a G28-1 single chain Fv binding domain from a ~~G28-1~~ hybridoma, wherein in the ~~connecting hinge~~ region said second cysteine residue is ~~replaced by~~ substituted with serine and said proline residue is ~~replaced by~~ substituted with serine, and wherein said heavy chain constant region comprises CH2 and CH3 domains from IgG₁.

96. (Previously presented) The protein of ~~claim 81~~ claim 77 wherein said single chain protein comprises a G28-1 single chain Fv binding domain from a ~~G28-1~~ hybridoma, wherein the ~~connecting hinge~~ region said first and second cysteine residues are ~~replaced by~~ substituted with serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁.

97. (Currently amended) The protein of ~~claim 81~~ claim 77 wherein said single chain protein comprises a FC2-2 single chain Fv binding domain from a ~~FC2-2~~ hybridoma, wherein in the ~~connecting hinge~~ region said ~~first, second, and third~~ cysteine residues are ~~replaced by~~ residue is substituted with serine and said proline residue is ~~replaced by~~

substituted with serine, and wherein said heavy chain constant region comprises CH2 and CH3 domains from IgG₁.

98. (Currently amended) The protein of ~~claim 81~~ claim 77 wherein said single chain protein comprises a UCL-1 single chain Fv binding domain ~~from a UCL-1 hybridoma~~, wherein in the ~~connecting hinge~~ region said ~~first, second, and third~~ cysteine residues ~~are replaced by~~ residue is substituted with serine and said proline residue is ~~replaced by~~ substituted with serine, and wherein said heavy chain constant region comprises CH2 and CH3 domains from IgG₁.

99. (Currently amended) The protein of ~~claim 81~~ claim 77 wherein said single chain protein comprises a 5B9 single chain Fv binding domain ~~from a 5B9 hybridoma~~, wherein in the ~~connecting hinge~~ region said ~~first, second, and third~~ cysteine residues ~~are replaced by~~ residue is substituted with serine and said proline residue is ~~replaced by~~ substituted with serine, and wherein said heavy chain constant region comprises CH2 and CH3 domains from IgG₁.

100. (Currently amended) The protein of ~~claim 81~~ claim 77 wherein said single chain protein comprises a 2H7 single chain Fv binding domain ~~from a 2H7 hybridoma~~, wherein in the ~~connecting hinge~~ region said ~~first, and second, and third~~ cysteine residues ~~are replaced by~~ substituted with serine ~~and said proline residue is replaced by~~ serine, and wherein said heavy chain constant region comprises CH2 and CH3 domains from IgG₁.

101. (Currently amended) The protein of ~~claim 81~~ claim 77 wherein said single chain protein comprises a 2H7 single chain Fv binding domain ~~from a 2H7 hybridoma~~, wherein in the ~~connecting hinge~~ region said first and second ~~and third~~ cysteine residues ~~are replaced by~~ substituted with serine and said proline residue is ~~replaced by~~ substituted with serine, and wherein said heavy chain constant region comprises CH2 and CH3 domains from IgG₁.

102. (Currently amended) The protein of ~~claim 81~~ claim 77 wherein said single chain protein comprises a G28-1 single chain Fv binding domain ~~from a G28-1 hybridoma~~, wherein in the ~~connecting hinge~~ region said first and second ~~and third~~ cysteine residues ~~are replaced by~~ substituted with serine and said proline residue is ~~replaced by~~ substituted with

serine, and wherein said heavy chain constant region comprises CH2 and CH3 domains from IgG₁.

103. (Currently amended) The protein of ~~claim 81~~ claim 77 wherein said single chain protein comprises a G28-1 single chain Fv binding domain ~~from a G28-1 hybridoma~~ comprising a ~~connecting hinge~~ region wherein said ~~second~~ third cysteine residue is ~~replaced by~~ substituted with serine and said proline residue is ~~replaced by~~ substituted with serine, and wherein said heavy chain constant region comprises CH2 and CH3 domains from IgG₁.

104. – 106. (Canceled)

107. (Currently amended) The protein of ~~claim 81~~ claim 77 wherein said single chain protein comprises a HD37 single chain Fv binding domain ~~from a HD37 hybridoma~~, wherein in the ~~connecting hinge~~ region said ~~first, second, and third~~ cysteine residues are ~~replaced by~~ residue is substituted with serine and said proline residue is ~~replaced by~~ substituted with serine, and wherein said heavy chain constant region comprises CH2 and CH3 domains from IgG₁.

108. (Currently amended) The protein of ~~claim 81~~ claim 77 wherein said single chain protein comprises a L6 single chain Fv binding domain, wherein in the ~~connecting hinge~~ region said ~~first, second, and third~~ cysteine residues are ~~replaced by~~ residue is substituted with serine and said proline residue is ~~replaced by~~ substituted with serine, and wherein said heavy chain constant region comprises CH2 and CH3 domains from IgG₁.

109. (Canceled)

110. (Currently amended) A single chain protein comprising the amino acid sequence of construct G28-2V_HL11S (SSC-P) H WCH2WCH3 as set out in SEQ ID NO: 329.

111. (New) The single chain protein of claim 1 wherein in the hinge region, the second cysteine is substituted with a serine, and a proline N-terminal to the third cysteine in the hinge is substituted with a serine.

112. (New) The single chain protein of claim 1 wherein in the hinge region, the first and second cysteines are substituted with a serine.